

Hidden diversity of micro-eukaryotes in Lake Baikal: a metagenomic approach

Mincheva E.V.^{1*}, Peretolchina T.E.¹, Kravtsova L.S.¹, Tupikin A.E.²,
Pudovkina T.A.¹, Ananyeva I.A.³, Triboy T.I.¹, Voylo M.A.¹, Bukin Yu.S.^{1,3}

¹ Limnological Institute, Siberian Branch of the Russian Academy of Sciences, Ulan-Batorskaya Str., 3, Irkutsk, 664033, Russia

² Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Lavrentiev Ave., 8, Novosibirsk, 630090, Russia

³ Irkutsk State University, Karl Marx Str., 1, Irkutsk, 664003, Russia

ABSTRACT. We have studied the diversity and composition of communities of micro-eukaryotes from two localities: area under anthropogenic impact near the town of Baikalsk and reference area, Irinda Bay, based on high-throughput ITS2 rDNA region sequencing. High taxonomic diversity of micro-eukaryotes has been revealed in both localities studied. However, micro-eukaryotic community from the area under anthropogenic impact is depressed, compared to reference area. Analysis of taxonomic diversity has revealed that community from the area under anthropogenic impact has a trend towards a decrease in diversity. Among the detected taxa, Alveolata, Stramenopiles and Fungi prevailed. Sørensen index of OTUs was 65%, which indicates the similarity of communities studied by taxonomic composition. The spectrum of Alveolata and Stramenopiles taxa identified in the area under the anthropogenic impact and the reference area was similar. The main differences were revealed in spectrum of Fungi taxa.

Keywords: micro-eukaryotes, metagenome, ITS2 rDNA, algae, Lake Baikal

1. Introduction

Although micro-eukaryotes have been investigated since the 17th century, they still remain understudied, especially in freshwater ecosystems (Debroas et al., 2017). Protists, Stramenopiles, Chromista, Cryptophyta and Fungi comprise micro-eukaryotes. Knowledge about the diversity of the micro-eukaryotic community in freshwater reservoirs is essential for predicting the ecosystem functioning.

The taxonomically and functionally diverse micro-eukaryotes communities have remained vastly unexplored due to the complexity of their identification, peculiarities of the life cycle, the presence of cryptic species, etc.

Currently, the development of high-throughput sequencing has allowed for a more full assessment of taxonomic diversity of communities, including even their hardly detectable members (Mueller et al., 2011; Debroas et al., 2017). Thus, using high-throughput sequencing methods, a large variety of micro-eukaryotes was discovered in freshwater ecosystems (Kammerlander et al., 2015).

Different fragments of 18S rDNA and internal transcribed spacer ITS1 are the most used molecular

genetic markers for metabarcoding studies of eukaryotic communities (Bokulich and Mills, 2013; Charvet et al., 2014; Kammerlander et al., 2015). However, recently it has been shown that ITS2 compared to ITS1 is more informative, especially, for a description of the taxonomic composition of fungal communities (Bazzicalupo et al., 2013; Banchi et al., 2018a; 2018b).

Recently, high-throughput sequencing of 18S rDNA was performed for analysis of micro-eukaryote diversity in Lake Baikal, and allowed to investigate the communities of the water column, bottom sediments, as well as green algae communities and their associated organisms (Yi et al., 2017; Mikhailov et al., 2019). These studies provided data on the presence of Fungi from various taxa: zygomycetes, glomeromycetes, chytridiomycetes, asco- and basidiomycetes, as well as allowed to describe the diversity of the SAR group (Stramenopiles, Alveolata, Rhizaria) in Lake Baikal.

During the past decade, researchers recorded the massive blooming of filamentous algae in Lake Baikal. In some parts of the lake, the changes in the dominant phyto-benthos species were observed (Kravtsova et al., 2012). In this regard, the study of micro-eukaryotes would be useful to identify species that may play an important role in these processes.

*Corresponding author.

E-mail address: elenakuznetsova01@gmail.com (E.V. Mincheva)

The aim of this study was to explore the taxonomic diversity of aquatic fungi and protists from the reference area and the area under anthropogenic impact at Lake Baikal using ITS2 marker.

2. Material and methods

The samples were collected in summer, 2018 from two localities of Lake Baikal: reference area, Irinda Bay (54°50'N; 109°40'E; the temperature of water was 12 °C, water transparency by Secchi disc was 6 m), and area with strong anthropogenic impact, near Baikalsk town (51°30'N; 104°15'E; the temperature of water was 11 °C, water transparency by Secchi disc was 3 m). The samples were collected at three depths according to vertical zonality (Izhboldina, 2007) of green alga distribution: 0 – 2 m, 2 – 5 m and 6 – 10 m.

DNA was extracted from the algae, their substrate and filtered water (free from invertebrates) according to the modified protocol described by Doyle and Dickson (1987). For each locality, 60 DNA samples were extracted and mixed for subsequent PCR.

ITS2 rDNA was amplified in PCR using the primers: ITS3_KYO2 F: 5'-GAT GAA GAA CGY AGY RAA-3' (Asemaninejad et al., 2016) and ITS4 R: 5'-TCC TCC GCT TAT TGA TAT GC-3' (White et al., 1990).

PCR and Illumina MiSeq paired-end sequencing were performed in the SB RAS Genomics Core Facility (ICBFM SB RAS, Novosibirsk).

The UPARSE pipeline (Edgar, 2013) was used to cluster contigs of all samples. Contigs with 97% similarity were classified into one operational taxonomic unit (OTU).

The representativeness OTUs (number of sequences per OTU) of species rank were used to calculate Shannon indices of community biodiversity (Shannon, 1948). Underestimated α -diversity was evaluated using Chao1 (Chiu et al., 2014). Calculations were performed using the R packages «vegan» (Dixon, 2003).

3. Results and Discussion

After stitching, trimming and chimera removing, total dataset consisted of 89134 reads, where micro-eukaryotes were represented by 9966 reads (3778 reads for Irinda Bay (reference area) and 6188 reads for Baikalsk city (area under anthropogenic impact)). The average length of the reads was 340 bp.

Sequence dataset was clustered into 144 OTUs of species rang, 123 OTUs of them occurred in Irinda Bay

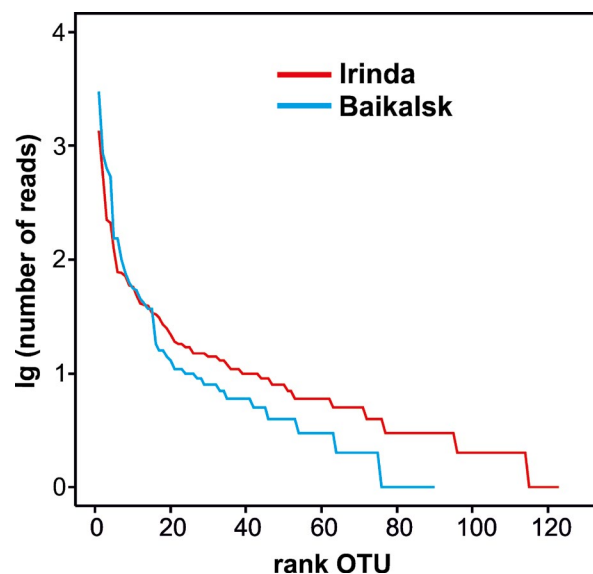


Fig. 1. Species abundance curves of the communities studied.

and 90 OTUs were found in Baikalsk (Supplementary Table can be found online at <https://github.com/barnsys/microeukaryoticcommunity>), both communities shared 69 OTUs. The index Chao1 showed that the taxonomic diversity of communities observed and predicted differed by < 10% (Table 1). This means that the amount of input data was sufficient to fully characterize taxonomic diversity (species richness) in each of the two communities studied.

Previous studies of micro-eukaryotic communities in Lake Baikal were performed using 454-sequencing, which allowed produce from several hundreds to 30000 reads per sample in average (Mikhailov et al., 2019). The total amount of reads included not only micro-eukaryotes but also the dominant phytoplankton and metazoa OTUs. Due to a relatively small number of reads, the number of underestimated OTUs of species rank in these studies was 10% or more. In our study, the Illumina MiSeq technology allowed production of a greater number of reads, due to which we obtained reliable estimates of species richness even for rare components of communities.

Shannon's and Evenness diversity indices of micro-eukaryotes communities from Irinda Bay were higher than for those near Baikalsk town (Table 1). The species abundance curve for the communities near Baikalsk town is significantly sharper than that of the Irinda Bay community. The analysis has indicated that micro-eukaryotic community from the area under anthropogenic impact near Baikalsk town is depressed compared to the reference area, Irinda Bay (Fig. 1).

Table 1. The diversity indices estimated for the communities studied

Sampling locations	Number of species	Chao1 index	Shannon index	Evenness
Irinda	123	124	2.88	0.59
Baikalsk	90	98	2.04	0.45

Sørensen similarity index of OTUs detected in the studied communities was 65%. In both micro-eukaryotic communities studied, Alveolata was the most numerous group (83–97%) (Fig. 2A). The diversity of Alveolata taxa from both localities was approximately equal, but their abundance was different (Fig. 2A). Among Alveolata, the representatives of Oligohymenophorea, in particular, Ciliata (*Vorticella*, *Pseudovorticella*), were the most abundant. Stramenopiles comprised 20% and 9% in communities from Irinda Bay and Baikalsk town, respectively (Fig. 2B). Stramenopiles from both communities were mostly presented by

diatom alga Bacillariophyceae (*Gomphonema* sp., *Didymosphenia geminata*, *Amphiprora* sp., *Fragilariopsis* sp.) and were more abundant in the community from the reference area of Irinda Bay (Fig. 2D). *D. geminata* was more representative in the reference area, whereas *Gomphonema* sp. was more abundant in the area with anthropogenic impact. Representatives of both genera are typical for the stony littoral zone of open Lake Baikal. Alveolata and Stramenopiles are key components of aquatic food webs both as a food source (e.g. diatoms) and as major consumers of bacterial biomass (e.g. ciliates) (Šlapeta et al., 2005).

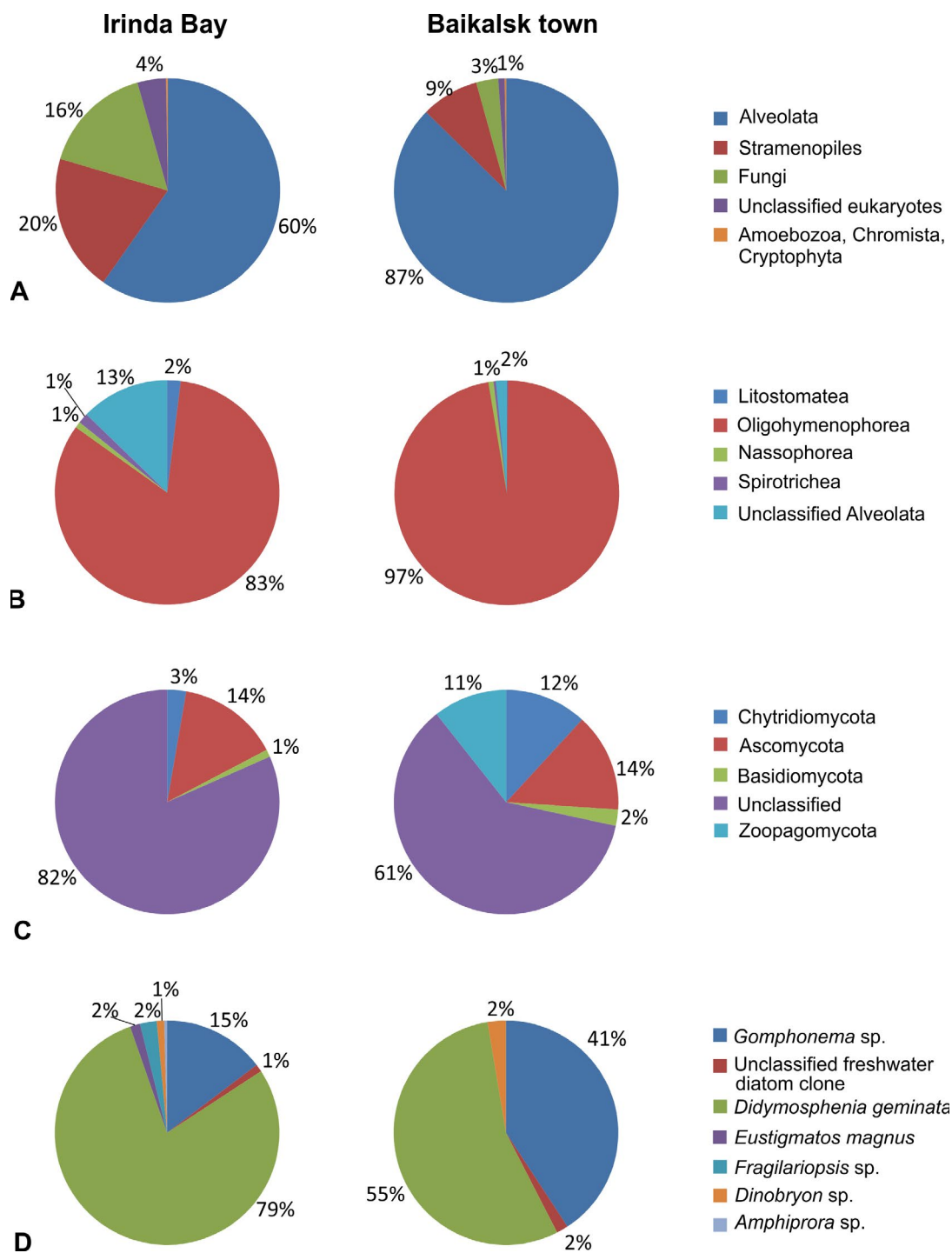


Fig. 2. Community structure of micro-eukaryotes from two localities of Lake Baikal. A – total micro-eukaryotic diversity from Irinda Bay and Baikalsk town; B – diversity of Alveolata from Irinda Bay and Baikalsk town; C – diversity of Fungi from Irinda Bay and Baikalsk town; D – diversity of Stramenopiles from Irinda Bay and Baikalsk town.

Fungi comprised 3–16% of the communities studied (Fig. 2A). Fungi contribute significantly to the organic matter decomposition and food web; they affect water quality and taxonomic composition of communities (Zhang et al., 2015; 2018). Fungi from Irinda Bay were more abundant. The bulk of the sequences obtained belonged to unclassified freshwater fungi, probably, due to a small number of ITS2 sequences for identified species of Fungi available through GenBank. The composition of communities from two localities was different. Among Ascomycota, saprotrophs or alga parasites, *Alternaria* sp. and *Cladosporium floccosum* were more abundant in the reference area. Representatives of phylum Zoopagomycota, which in general are parasites of micro-metazoan, were observed only in Baikalsk (Fig. 2C). Members of the Chytridiomycota are known to be widespread in freshwater ecosystems, where they act as parasites and decomposers (Johnson et al., 2006; Kagami et al., 2012). Chytridiomycota were more abundant in the area under anthropogenic impact and were mostly represented by *Rhizophidium*, members of which are phytopathogens and alga parasites (Letcher et al., 2004).

Conclusions

Taxonomic diversity of micro-eukaryotes in the reference area of Irinda Bay was higher than that in the area under anthropogenic impact. Taxonomic composition of Alveolata and Stramenopiles from the communities studied was similar, and the main differences were related with the abundance of their taxa. Communities from different areas mostly differed in taxonomic composition of Fungi. The community from the reference area lacked representatives of Zoopagomycota, whereas they are abundant in the region under anthropogenic impact. In addition, in the latter locality Chytridiomycota were mostly represented by *Rhizophidium*, members of which are phytopathogens and alga parasites. Representatives of this genus may be recommended as markers for monitoring of the stability of freshwater alga community.

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References

- Asemaninejad A., Weerasuriya N., Gloor G.B. et al. 2016. New primers for discovering fungal diversity using nuclear large ribosomal DNA. *PloS one* 11. DOI: 10.1371/journal.pone.0159043
- Banchi E., Ametrano C.G., Stanković D. et al. 2018a. DNA metabarcoding uncovers fungal diversity of mixed airborne samples in Italy. *PloS one* 13. DOI: 10.1371/journal.pone.0194489
- Banchi E., Stankovic D., Fernández-Mendoza F. et al. 2018b. ITS2 metabarcoding analysis complements lichen mycobiome diversity data. *Mycological Progress* 17: 1049–1066. DOI: 10.1007/s11557-018-1415-4
- Bazzicalupo A.L., Bálint M., Schmitt I. 2013. Comparison of ITS1 and ITS2 rDNA in 454 sequencing of hyperdiverse fungal communities. *Fungal Ecology* 6: 102–109. DOI: 10.1016/j.funeco.2012.09.00
- Bokulich N.A., Mills D.A. 2013. Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. *Applied and Environmental Microbiology* 79: 2519–2526. DOI: 10.1128/AEM.03870-12
- Charvet S., Vincent W.F., Lovejoy C. 2014. Effects of light and prey availability on Arctic freshwater protist communities examined by high-throughput DNA and RNA sequencing. *FEMS Microbiology Ecology* 88: 550–564. DOI: 10.1111/1574-6941.12324
- Chiu C.H., Wang Y.T., Walther B.A. et al. 2014. An improved nonparametric lower bound of species richness via a modified good–turing frequency formula. *Biometrics* 70: 671–682. DOI: 10.1111/biom.12200
- Debroas D., Domaizon I., Humbert J.F. et al. 2017. Overview of freshwater microbial eukaryotes diversity: a first analysis of publicly available metabarcoding data. *FEMS Microbiology Ecology* 93: 1–14. DOI: 10.1093/femsec/fix023
- Dixon P. 2003. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14: 927–930. DOI: 10.1111/j.1654-1103.2003.tb02228.x
- Doyle J.J., Dickson E.E. 1987. Preservation of plant samples for DNA restriction endonuclease analysis. *Taxon* 36: 715–722. DOI: 10.2307/1221122
- Edgar R.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* 10: 996. DOI: 10.1038/nmeth.2604
- Izhboldina L.A. 2007. Guide and key to benthonic and periphyton algae of Lake Baikal (meio – and macrophytes) with short information about their ecology. Novosibirsk: Nauka-Center. (in Russian)
- Johnson P.T., Longcore J.E., Stanton D.E. et al. 2006. Chytrid infections of *Daphnia pulex*: development, ecology, pathology and phylogeny of *Polycaryum laeve*. *Freshwater Biology* 51: 634–648. DOI: 10.1111/j.1365-2427.2006.01517.x
- Kagami M., Amano Y., Ishii N. 2012. Community structure of planktonic fungi and the impact of parasitic chytrids on phytoplankton in Lake Inba, Japan. *Microbial Ecology* 63: 358–368. DOI: 10.1007/s00248-011-9913-9
- Kammerlander B., Breiner H.W., Filker S. et al. 2015. High diversity of protistan plankton communities in remote high mountain lakes in the European Alps and the Himalayan mountains. *FEMS Microbiology Ecology* 91. DOI: 10.1093/femsec/fiv010
- Kravtsova L.S., Izhboldina L.A., Khanaev I.V. et al. 2012. Disturbances of the vertical zoning of green algae in the coastal part of the Listvenichnyi gulf of Lake Baikal. *Doklady Biological Sciences* 447: 350–352. DOI: 10.1134/S0012496612060026
- Letcher P.M., Powell M.J., Chambers J.G. et al. 2004. Phylogenetic relationships among *Rhizophidium* isolates from North America and Australia. *Mycologia* 96: 1339–1351. DOI: 10.1080/15572536.2005.11832883
- Mikhailov I.S., Zakharova Y.R., Bukin Y.S. et al. 2019. Co-occurrence networks among bacteria and microbial eukaryotes of Lake Baikal during a spring phytoplankton bloom. *Microbial Ecology* 77: 96–109. DOI: 10.1007/s00248-018-1212-2

Mueller G.M., Foster M.S., Bills G.F. 2011. Biodiversity of fungi: inventory and monitoring methods. New York: Academic.

Shannon C.E. 1948. A mathematical theory of communication. *Bell Labs Technical Journal* 27: 379–423. DOI: 10.1002/j.1538-7305.1948.tb01338.x

Šlapeta J., Moreira D., López-García P. 2005. The extent of protist diversity: insights from molecular ecology of freshwater eukaryotes. *Proceedings of the Royal Society B: Biological Sciences* 272: 2073–2081. DOI: 10.1098/rspb.2005.3195

White T.J., Bruns T.D., Lee S.B. et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J. et al. (Eds.), *PCR protocols: a guide to methods and applications*. New York, pp. 315–322.

Yi Z., Berney C., Hartikainen H. et al. 2017. High-throughput sequencing of microbial eukaryotes in Lake Baikal reveals ecologically differentiated communities and novel evolutionary radiations. *FEMS Microbiology Ecology* 93. DOI: 10.1093/femsec/fix073

Zhang H., Huang T., Chen S. 2015. Ignored sediment fungal populations in water supply reservoirs are revealed by quantitative PCR and 454 pyrosequencing. *BMC Microbiology* 15: 44. DOI: 10.1186/s12866-015-0379-7

Zhang H., Jia J., Chen S. et al. 2018. Dynamics of bacterial and fungal communities during the outbreak and decline of an algal bloom in a drinking water reservoir. *International Journal of Environmental Research and Public Health* 15: 361. DOI: 10.3390/ijerph15020361